Fisheries Journal, 14(3), 1350-1359 (2024)
http://doi.org/10.29303/jp.v14i3.893

THE SPREAD OF INFECTIOUS MYONECROSIS VIRUS (IMNV) IN CARRIERS BIOTA AND WATERS AT SHRIMP POND IN ANYER AND CARITA COASTAL, BANTEN PROVINCE

Sebaran Infectious Myonecrosis Virus (IMNV) pada Biota *Carrier* dan Perairan di Sekitar Tambak Udang di Wilayah Pesisir Anyer dan Carita, Provinsi Banten

Indra Gumay Yudha[1*], Muhamad Fatin Choiri[1], Indriasih[2], Qadar Hasani[1], Anma Hari Kesuma[3]

[1]Aquatic Resources Study Program, Department of Fisheries and Marine Science, Lampung University, [2]Fish and Environmental Health Testing Center, Serang, [3]Marine Science Study Program, Department of Fisheries and Marine Science, Lampung University

Sumantri Brojonegoro Street no. 01, Gedong Meneng, Rajabasa, Bandar Lampung City 35141

*Corresponding Author: indra_gumay@yahoo.com

(Received July 2th 2024; Accepted September 19th 2024)

ABSTRACT

This research was motivated by shrimp ponds in the coastal areas of Anyer and Carita that were not by the regional regulations and their existence was not monitored. Waste from shrimp pond activities will pollute the surrounding waters. The waste can contain pathogens such as infectious myonecrosis virus (IMNV) which are dangerous and threaten other shrimp farmers. The purpose of this study was to identify the presence of infectious myonecrosis virus (IMNV) in the aquatic environment and carrier biotas around shrimp ponds in the coastal areas of Anyer and Carita. IMNV detection used samples of hermit crabs, barnacles, and water using the real-time quantitative reverse transcriptase-PCR (qRT-PCR) method. The qRT-PCR results showed that 6 samples detected IMNV spread over 4 locations with Ct values 36.97 (station 1.1), 35.95 (station 5.1), 35.59 (station 5.2), 35.05 (station 6.1), 35.22 (station 6.2), and 35.54 (station 7.1).

Keywords: Anyer, Carita, IMNV, qRT-PCR, Surveillance.

ABSTRAK

Penelitian ini dilatarbelakangi oleh keberadaan tambak udang di wilayah pesisir Anyer dan Carita tidak sesuai dengan peraturan daerah yang berlaku dan tidak terawasi keberadaannya. Limbah hasil kegiatan tambak udang dapat mencemari perairan sekitar. Limbah tersebut dapat mengandung patogen seperti *infectious myonecrosis virus* (IMNV) yang berbahaya dan mengancam pembudi daya udang lainnya. Tujuan penelitian ini adalah untuk mengidentifikasi keberadaan *infectious myonecrosis virus* (IMNV) pada lingkungan perairan dan *carrier* yang terdapat di sekitar tambak udang di wilayah pesisir Anyer dan Carita. Deteksi IMNV menggunakan sampel kelomang, teritip, dan air dengan metode *Real-time quantitative Reverse Transcriptase*-PCR (qRT-PCR). Hasil qRT-PCR menunjukkan 6 sampel terdeteksi IMNV

yang tersebar di 4 lokasi dengan nilai Ct 36,97 (stasiun 1.1), 35,95 (stasiun 5.1), 35,59 (stasiun 5.2), 35,05 (stasiun 6.1), 35,22 (stasiun 6.2), dan 35,54 (stasiun 7.1).

Kata kunci: Anyer, Carita, IMNV, qRT-PCR, Surveilans.

INTRODUCTION

The Anyer and Carita coasts are one of the areas with aquaculture potential. Management for this area is contained in Regional Regulation (Perda) Number 5 of 2020 concerning spatial planning for the Serang Regency and Regional Regulation (Perda) Number 2 of 2020 concerning spatial planning for the Pandeglang Regency. One of these regional regulations regulated spatial planning for the Anyer and Carita coastal areas, such as brackish water hatcheries. However, in this area there are many shrimp pond, meaning that the existence of these ponds did not comply with their intended purpose and violates applicable regional regulations. With this in mind, its existence was not monitored so that it can cause water pollution by production waste in the form of leftover shrimp feed, feces, and dead organisms (Ge *et al*., 2019). This waste then affects environmental components, such as biological, physical, and chemical of water quality. Shrimp wastewater also contains pathogens such as bacteria and viruses that can be transmitted (León-Cañedo et al., 2019). One of the pathogens that is dangerous and detrimental to aquatic ecosystems is the infectious myonecrosis virus (IMNV). If this virus spreads to the waters, it will pollute the aquatic ecosystem and threaten shrimp hatchery farms.

IMNV had threatened the shrimp farming industry in Indonesia, and even the world. This virus can cause death in shrimp up to 40-70% of the population (OIE, 2009). IMNV transmission can occur horizontally through water and carrier organisms (OIE, 2021). Until now no treatment or vaccine can be used to control the spread of IMNV (OIE, 2019), so the efforts that can currently be made to suppress the spread are prevention by carrying out surveillance (observation) of the presence of this disease. This was done to obtain information on the presence of IMNV in the surrounding waters. Therefore, this research was carried out to detect the presence of IMNV in the aquatic environment and carriers around shrimp ponds in the Anyer and Carita coastal areas.

RESEARCH METHODS

The research method used was descriptive with a quantitative approach. According to Murdiyanto (2020), quantitative research is based on a framework of thought that greatly determines the clarity and validity of the overall research process. Through the description in the framework of thought, researchers can comprehensively explain the variables studied and the theories from which the variables are derived, and why only those variables are studied. Furthermore, Murdiyanto (2020) stated that quantitative methods, especially survey and experimental methods, are used when the research problem is clear, want to obtain broad information from a population, want to obtain accurate data, and the research is based on empirical and measurable phenomena. Quantitative descriptive research was expected to explain in detail the purpose of this research, namely to discover and describe environmental conditions and the presence of infectious myonecrosis virus (IMNV) in the waters around shrimp ponds in the coastal areas of Anyer and Carita. The research was conducted in August-September 2022 in the coastal waters of Anyer (Serang Regency) and Carita (Pandeglang Regency), Banten Province, where there were shrimp farming activities. The sampling method used a purposive sampling method by determining the research location based on certain considerations, including the location of the shrimp pond which was close to the shrimp hatchery location. Sampling was carried out at 7 stations with 2 points at each station. Stations

1 to 2 were in the Anyer coastal area, whereas stations 3 to 7 were in the Carita coastal area. Samples were taken from the beach adjacent to the inlet and outlet of the shrimp pond in the form of hermit crabs, barnacles, and water. The number of samples was taken at station 1, station 2, station 5, and station 6 point 2 was 6. At station 3, station 4, station 6 point 1, and station 7 only 4 samples were taken due to the unavailability of barnacle biota. So the number of samples obtained in the first and second collections was 35 each. Thus, in this study, a total of 70 samples were tested.

Data on water quality parameters measured *in situ* were DO, temperature, salinity and pH, while TOM parameters were tested in the laboratory. This research procedure had a preparatory stage including preparation of tools and on-site surveys (*in situ*). The implementation stage included determining the location, taking samples, measuring water quality, and diagnosing the virus using qRT-PCR. The qRT-PCR test consisted of five stages, i.e sample preparation, RNA extraction, measurement of RNA concentration and purity, amplification, and interpretation of results. The qRT-PCR test consisted of five stages based on SNI 7662-3: 2021 including sample preparation, RNA extraction, measurement of RNA concentration and purity, amplification, and interpretation of results. The amplification process used materials Real IQ 2000 IMNV kit started by making an IMNV master mix with components for 1 reaction, namely RT-PCR premix 22 µL, RT-enzyme 1 µL, IQ enzyme 2 µL, and RNA template 2 µL. Also prepared were 6 positive controls with serial dilutions (10^6 copies/µL - 10 copies/µL) and one negative control/NTC (non-template control). All amplification master mix materials were homogenized and distributed 23 µL into each optical qPCR plate, then added *template* test samples, 6 serial positive controls, and 1 serial negative control 2 µL. The amplification profile used was 48 °C for 5 minutes (reverse transcriptase), 96 °C for 20 seconds (initial denaturation), 95 °C for 1 second 40 cycles and 60 °C for 20 seconds 40 cycles (amplification) .

RESULTS

Physical and chemical of water quality

The results of measuring the physical and chemical parameters of the waters around the ponds in the Anyer and Carita coastal areas were presented in Table 1 (1^{st} sampling) and Table 2 (2^{nd} sampling).

Table 1. Physical and chemical parameters of the 1^{st} sampling

No	Station	DO (mg/L)	Temperature (ºC)	Salinity (ppt)	pH	TOM (mg/L)
1	1.1	8.94	25.3	36	7.61	16.65
2	1.2	9.55	25.5	34	7.49	20.10
3	2.1	9.96	26.4	33	7.37	17.23
4	2.2	8.63	24.8	32	7.31	17.23
5	3.1	9.40	25.0	31	7.64	21.82
6	3.2	9.56	25.4	30	7.60	15.50
7	4.1	9.70	26.0	32	7.77	20.10
8	4.2	9.97	26.5	30	7.55	20.10
9	5.1	9.40	25.3	25	7.53	15.50
10	5.2	9.87	25.3	28	7.52	14.36
11	6.1	9.95	26.3	32	7.75	21.25
12	6.2	9.83	25.9	32	7.72	20.67
13	7.1	9.55	26.0	31	8.00	15.50
14	7.2	9.37	25.6	31	7.72	20.67

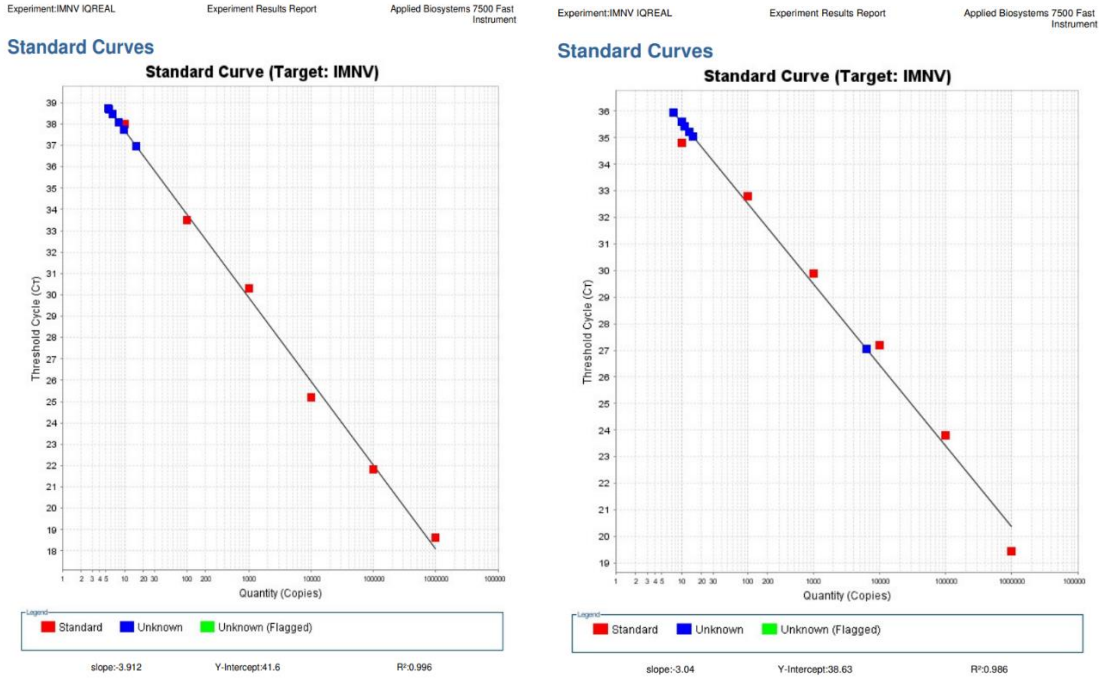
Table 2. Physical and chemical parameters of the 2nd sampling

No	Station	DO (mg/L)	Temperature (ºC)	Salinity (ppt)	pH	TOM (mg/L)
1	1.1	7.11	24.4	31	9.05	14.04
2	1.2	7.50	24.4	31	9.06	14.62
3	2.1	7.84	23.8	31	8.34	14.04
4	2.2	7.27	23.6	30	8.27	14.62
5	3.1	7.44	23.5	30	8.36	11.70
6	3.2	7.85	23.4	30	8.31	14.04
7	4.1	7.32	23.9	31	8.65	15.79
8	4.2	7.44	23.3	31	8.55	12.28
9	5.1	7.34	23.6	31	8.33	14.62
10	5.2	7.30	23.8	17	8.47	9.36
11	6.1	7.72	24.1	24	7.83	11,11
12	6.2	7.22	24.0	23	7.90	9.94
13	7.1	7.38	24.0	22	8.55	11.70
14	7.2	7.33	24.1	22	8.44	15.79

IMNV testing using qRT-PCR

IMNV testing used the quantitative reverse-transcriptase (qRT-PCR) method using test samples in the form of crustaceans and water. A total of 35 test samples with two replications were used in this research. The type of hermit crab used as a test sample was the genus of Clibanarius, while the barnacles was the genus Chthamalus and Tetraclita.

The results of IMNV virus detection using the qRT-PCR method (Figure 1 and Table 3) showed data in the form of Ct values which will determine whether the sample was positive/negative for IMNV. The Ct value in the first sampling ranged from 35.05-35.95 and in the second sampling a Ct value of 36.97 was detected. Samples are declared positive for IMNV virus when they have a Ct value <37. According to Bordon *et al*., (2020) the Ct value is inversely proportional to the number of target RNA copies in the sample, the lower the Ct value, the greater the number of target RNA copies in the sample and vice versa.



a). First sampling (August 2022) b). Second sampling (September 2022)

Figure 1. Standard curve of IMNV test results using qRT-PCR

Table 3. Results of qRT-PCR testing for the presence of IMNV

1st take			2nd take		
Sample	Station	*Cycle Threshold*(Ct)	Sample	Station	*Cycle Threshold*(Ct)
Hermit crabs	1.1	Undetermined	Hermit crabs	1.1	Undetermined
	1.2	Undetermined		1.2	Undetermined
	2.1	Undetermined		2.1	Undetermined
	2.2	Undetermined		2.2	Undetermined
	3.1	Undetermined		3.1	Undetermined
	3.2	Undetermined		3.2	Undetermined
	4.1	Undetermined		4.1	Undetermined
	4.2	Undetermined		4.2	Undetermined
	5.1	Undetermined		5.1	Undetermined
	5.2	Undetermined		5.2	Undetermined
	6.1	Undetermined		6.1	Undetermined
	6.2	Undetermined		6.2	Undetermined
	7.1	Undetermined		7.1	Undetermined
	7.2	Undetermined		7.2	Undetermined
Barnacles	1.1	Undetermined	Barnacles	1.1	Undetermined
	1.2	Undetermined		1.2	Undetermined
	2.1	Undetermined		2.1	Undetermined
	2.2	Undetermined		2.2	Undetermined
	5.1	Undetermined		5.1	Undetermined
	5.2	Undetermined		5.2	Undetermined
	6.2	Undetermined		6.2	Undetermined
	7.1	Undetermined		7.1	Undetermined
	7.2	Undetermined		7.2	Undetermined
Sea water	1.1	Undetermined	Sea water	1.1	36.97
	1.2	Undetermined		1.2	Undetermined
	2.1	Undetermined		2.1	Undetermined
	2.2	Undetermined		2.2	Undetermined
	3.1	Undetermined		3.1	Undetermined
	3.2	Undetermined		3.2	Undetermined
	4.1	Undetermined		4.1	Undetermined
	4.2	Undetermined		4.2	Undetermined
	5.1	35.95		5.1	Undetermined
	5.2	35.59		5.2	Undetermined
	6.1	35.05		6.1	Undetermined
	6.2	35.22		6.2	Undetermined
	7.1	35.44		7.1	Undetermined
	7.2	Undetermined		7.2	Undetermined

Description: Undetermined= IMNV not detected (IMNV negative)

PAMKI (2020), stated that the Ct value <29 of the sample was detected as strongly positive, namely that there was a large amount of target nucleic acid, the Ct value between 30-37 was moderately positive, and 38-40 was weakly positive or there was the possibility of

contamination from the environment. Based on this, the six of water samples in the study were moderately positive.

The qRT-PCR result data was then processed in the form of an IMNV distribution map using Arcgis software. In Figure 1 the red dots on the distribution map showed the locations where IMNV was detected. The distribution of IMNV showed that positive samples were detected in 4 locations, namely, station 1, station 5, station 6, and station 7. Station 1 was located in Serang Regency, i.e Florida Beach, Sindang Laya Village. The other three stations were located in Pandeglang Regency, i.e Sambolo Beach, Sukarame Village (station 5), Carita Beach, Banjarmasin Village (station 6), and Karang Tumpeng Beach, Pejamben Village (station 7).

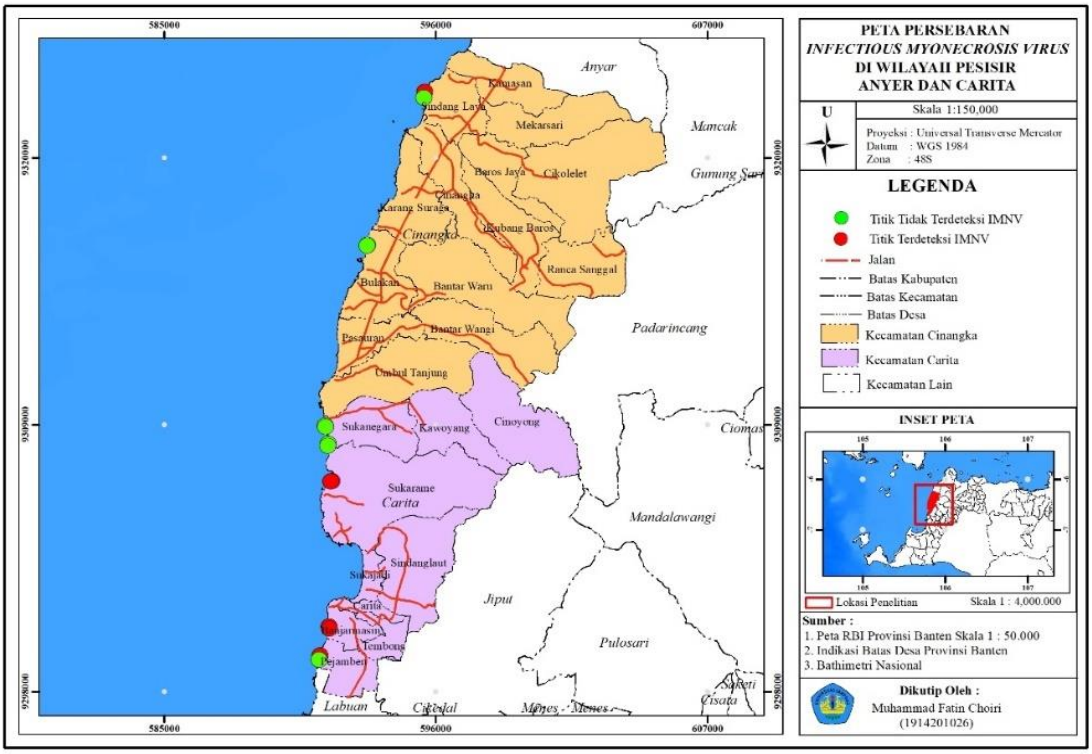


Figure 2. Distribution of IMNV in the coastal areas of Anyer and Carita, Banten

DISCUSSION

The dissolved oxygen value of the waters in the first sampling (Table 1) ranged from 8.63-9.97 mg/L and in the second sampling (Table 2) ranged from 7.11-7.85 mg/L. The dissolved oxygen concentration in the first sampling was higher compared to dissolved oxygen in the second sampling occurred due to differences in data collection time. The first data collection was carried out during the day so that with the abundant availability of sunlight, more photosynthesis processes occurred and more oxygen was produced. On the other hand, in the second data collection the dissolved oxygen was lower because it was carried out in the afternoon so that the photosynthesis process was reduced. This high dissolved oxygen level was influenced by various things, one of which was the process of photosynthesis and oxygen diffusion from the atmosphere, where the dissolved oxygen value in water usually had a range of between 6-14 mg/L (Connell *et al*., 1995). Rouf *et al.* (2022) stated that dissolved oxygen in waters ranged from 6-8.6 mg/L, which was relatively lower when compared with research results.

The results of observations of the water temperature varied quite widely, ranged from

25-26.5ºC in the first sampling and 23.3-24.4 ºC in the second sampling. Variations in water temperature in waters were influenced by several factors, such as atmospheric conditions, weather, and the intensity of sunlight entering the sea (Officer, 1976). In the first sampling, the water temperature was relatively higher than in the second sampling. This happened because the first data collection was carried out during the day so the temperature was relatively higher due to exposure to sunlight. The second collection was carried out in the afternoon so that sunlight was reduced. This was in accordance with Mark's (1995) statement that the temperature in a body of water is influenced by the length of sunlight. The temperature value in these waters was still within the normal range for marine waters in general, namely around 20-30 ºC (Khan & Rajshekhar, 2020). The sea water quality standard for marine biota is based on Government Regulation Number 22 of 2021, i.e 28-32 ºC. When compared with these criteria, the temperature at this research location was still considered normal and still within tolerance limits to support the life of marine biota.

The salinity values obtained in two repetitions ranged from 25-36 ppt in the first sampling and 17-31 ppt in the second sampling. Kinne (1964) stated that seawater had normal salinity values ranging from 33-37 ppt with an average value of around 35 ppt. According to Nasreen (2022), seawater salinity values mostly ranged between 34-36 ppt. However, the salinity values at several stations had a fairly low range and less than 30 ppt. The low salinity levels at several stations were influenced by several things such as rain and the inflow of river water. This was by Nasreen's (2022) statement that the salinity value of seawater depended on the volume of freshwater input from rivers and rain.

Observation results at stations 5 and 7 showed that at this location the salinity is low, ≤ 25 ppt, while at station 1 was higher (36 ppt). This happened because the sampling locations at stations 5 and 7 were near a river that emptied into the sea. According to Durack *et al*. (2014) the salinity of seawater adjacent to river flows had low salinity levels because it was influenced by freshwater carried by river flows, resulting in dilution of seawater, but salinity levels were still within normal salinity limits. This was following Nasreen's (2022) statement that salinity levels adjacent to estuary areas were low. Meanwhile, station 1 had higher salinity levels because at the time of sampling the weather conditions were sunny and the sea water was high tide. Hot weather conditions can increase the rate of evaporation of seawater so that the salinity level of sea water became more concentrated (Nasreen, 2022).

The pH value in the first sampling ranged from 7.31-8 with the lowest value at station 2.2 and the highest at station 7.1. In the second sampling, the pH value ranged between 7.83 and 9.06 with the lowest value at station 6.1 and the highest at station 1.2. The high pH values at several stations occurred due to shrimp farming activities which dispose of waste directly into the waters. Based on seawater quality standards through Government Regulation (PP RI) Number 22 of 2021, the pH value ranges from 7 to 8.5. Thus, the pH value at the second sampling at stations 1 and 4 exceeded the seawater quality standard limit. In general, the pH value of seawater ranged between 7.6 and 8.3 (Khan & Rajshekhar, 2020).

The organic matter content at the research location was observed through the content of the total organic matter (TOM). TOM in the waters around shrimp ponds in the coastal areas of Anyer and Carita varied quite widely, ranging from 14.36-21.82 mg/L in the first sampling and 9.36-15.79 mg/L in the second sampling. In the first sampling, the lowest value was at station 2.1 and the highest at station 3.1. Meanwhile, in the second sampling, the lowest TOM was at station 5.2 and the highest at station 4.1 and station 7.2. The TOM content showed the total value of the material organics in waters originating from detritus, phytoplankton or other biota waste products from the decomposition process by microorganisms (Heilskov & Holmer, 2001). According to Haryono *et al.*, (2021), total organic matter in waters was an accumulation of organic particles consisted of dissolved, suspended and colloidal organic matter. However,

TOM levels in waters should not be excessive because it can caused eutrophication. According to Yoswaty *et al.*, (2020) TOM levels with the highest value of 34.89 mg/L were within the normal range. Based on this statement, the TOM in this study was still within normal limits.

One of the causes of viruses spreading in waters is degradation of the aquatic environment (Walker & Mohan, 2009). Viruses, one of which is IMNV, will develop in waters when there is an interaction between a poor environment, the presence of IMNV, and weak host conditions (Anderson, 1974). In this case, in the Anyer-Carita coastal area, IMNV was detected in several locations. A total of 6 samples out of 70 samples or around 8.5% of samples detected by IMNV at 4 stations out of 7 stations or 57% of observation stations. Six samples of IMNV positive were water samples and no hermit crab and barnacle samples. This happened because the environmental conditions in the Anyer and Carita coastal areas were in good condition as seen from the physical and chemical of water quality parameters (Tables 1 and 2). This meant that environmental conditions were not stressful enough for the host (hermit crabs and barnacles) so that IMNV cannot infect the host. It was in accordance with the statement by Jha *et al* (2021) that this virus depended on host stress, and will be deadly when there were sudden changed in extreme water quality parameters such as salinity, pH, temperature and dissolved oxygen. The dependence of viruses on stressed hosts related to the nature of viruses, namely obligate cellular parasites that always need a host to reproduce (Jacquet *et al*., 2010). IMNV detected in water samples indicated the presence of the pathogen (IMNV) in those waters. Thus, it necessary to be aware that if there a decrease in the carrying capacity of the environment, it was not impossible that a disease outbreak will occur in the area detected by IMNV.

In general, the presence of viruses in waters is very abundant. The average abundance of viruses in waters is 10^7 particles mL^{-1} (Jacquet *et al*., 2010). This abundance is equivalent to 1030 viruses in aquatic ecosystems or around 90% of the total abundance of planktonic particles in waters are viruses (Suttle, 2007). The abundance of viruses in a body of water is directly proportional to the productivity of that water's ecosystem (Jacquet *et al*., 2010).

Based on IMNV detected in several areas and observations of water quality conditions, shrimp pond activities had quite an impact on the surrounding environment. Waste from shrimp pond that was thrown directly into the surrounding environment polluted the waters. This was indicated by the discovery of IMNV in water samples and the decline in water quality conditions at several observation stations. Thus, the results of this research can be a reference for policymakers regarding spatial planning to prevent increasingly severe pollution, as well as a source of information for cultivators to be alert to detected disease attacks.

CONCLUSION

Based on research conducted in the coastal areas of Anyer and Carita, Banten Province, it can be concluded that infectious myonecrosis virus (IMNV) was detected at 4 observation stations, i.e at Florida Beach, Sambolo Beach, Karang Tumpeng Beach, and Carita Beach. A total of 6 seawater samples out of 70 samples were detected by IMNV, while IMNV was not detected in carrier biota, either barnacles or hermit crabs.

ACKNOWLEDGEMENT

This research was carried out well because of the cooperation and provision of laboratory facilities from BPKIL Serang. For that, the authors would like to thank the Head of the Balai Pengujian Kesehatan Ikan dan Lingkungan (BPKIL) Serang and his staff.

REFERENCE

Bordon, J., Chung, D., Krishnan, P., Carrico, R., Ramirez J.A. (2020). The importance of cycle threshold values in the evaluation of patients with persistent positive PCR for SARS-Cov2: Case study and brief review. *Journal of Respiratory Infections*. 4(1): 1-5.

Connell, W.D., Miller, G.J. (1995). *Chemistry and Ecotoxicology of Pollution*. Translation, University of Indonesia Publishers. Jakarta.

Durack, P. K., Wijffels, S. E., Glecker, P. J. (2014). Long-term sea-level change revisited: the role of salinity. *Environmental Research Letters*. 9: 1-11.

Ge, J., Zhang, Y., Heo, Y. J., Park, S. J. (2019). Advanced design and synthesis of composite photocatalysts for remediation of wastewater. *A Review Catalysts*. 9(2):122-132.

Haryono, F.E.D., Divine, Z.Y., Dewi, R. (2021). Investigation of total organic matter (TOM) content during high and low water in inter-tidal zone sediment at Teluk Penyu Coast, Cilacap, Indonesia. *IOP Conf. Series: Earth and Environmental Science.* 746: 1-6

Heilskov, A. C., Holmer, M. (2001). Effects of benthic fauna on organic matter mineralization in fish-farm sediments: importance of size and abundance. *ICES J. of Marine Science.* 58: 427–434.

Jacquet, S., Miki, T., Noble, R., Peduzzi, P., Wilhelm, S. (2010). Viruses in aquatic ecosystems: important advances of the last 20 years and prospects for the future in the field of microbial oceanography and limnology. *Advances in Oceanography and Limnology*. 1(1): 97-141.

Kalangi, N.I.P., Mandagi, A., Masengi, K.W.A., Luasunaung, A., Panggaliga, F.P.T., Iwata, M. (2013). Distribution of temperature and salinity in Manado Bay, *J. Tropical Fisheries and Marine Affairs.* 9(2): 71-75.

Khan, Z. H., Rajshekhar, A. (2020). Relation among temperature, salinity, pH, DO of seawater quality. *Technium*. 2(4): 39-45.

Kinne, O. (1964). The effects of marine temperature and salinity on marine and brackish water animals. in salinity and temperature combinations. *Oceanography and Marine Biology Annual Review*, 2(2): 281-339.

León-Cañedo, J. A., Alarcón-Silvas, S. G., Fierro-Sañudo, J. F., de Oca, Partida-Ruvalcaba, L., Diaz-Valdés, T., Páez-Osuna, F. (2019). Mercury and other trace metals in lettuce (Lactuca sativa) grown with two low-salinity shrimp effluents: accumulation and human health risk assessment. *Sci Total Environ*. 650(2): 2535-2544.

Mark, A.S., Steven, H., Weisborth, C.L., Franklin, S. (1995). *Seawater Chapter 2: Temperature in the Oceans.* Butterworth-Heinemann. Britain: 14-28

Murdiyanto, E. 2020. *Metode Penelitian Kualitatif (Teori dan Aplikasi Disertai Contoh Proposal)*. Universitas Pembangunan Nasional Veteran Yogyakarta Press. Yogyakarta.

Nasreen, M. (2022). Ocean salinity. *International Journal for Modern Trends in Science and Technology*. 8(1): 296-302.

Office International des Epizooties (OIE). (2009). Infection With Infectious Myonecrosis Virus. Manual of Diagnostics for Aquatic Animals. Chapter 2.2.3.

Office International des Epizooties (OIE). (2019). Infection With Infectious Myonecrosis Virus. Manual of Diagnostics for Aquatic Animals. Chapter 2.2.5.

Office International des Epizooties (OIE). (2021). Infection With Infectious Myonecrosis Virus. Manual of Diagnostics for Aquatic Animals. Chapter 2.2.5.

Officer, C.B., (1976). *Physical Oceanography of Estuaries and Associated Coastal Waters*. John Willey and Sons. New York.

PAMKI (2020). *Clinical Meaning of Cycle Threshold (Ct) Values in Real Time RT-PCR Examination Results.* Indonesian Association of Clinical Microbiology Specialists. Jakarta.

Republic of Indonesia Government Regulations. (2021). Government Regulation No. 22

concerning Implementation of Environmental Protection and Management for Marine Biota. Jakarta.

Rouf, M.A., Islam, Md.J., Roknuzzaman, Md., Siddique, Md.N., Golder, Md.R. (2022). Vertical profile of dissolved oxygen and associated water variables in the Pasur-Rupsha estuary of Bangladesh. *Heliyon*. 8(10): 1-8

SNI 7662-3. (2021). Detection of Infectious Myonecrosis Virus (IMNV) - Part 3: Quantitative real-time Reverse Transcription - Polymerase Chain Reaction (qRT- PCR) method using a hydrolysis probe. National Standardization Agency. Jakarta.

Walker, P. J., Mohan, C. V. (2009). Viral disease emergence in shrimp aquaculture: origins, impact and the effectiveness of health management strategies. *Reviews in Aquaculture.* 1: 125-154.

Yoswaty, D., Amin, B., Nursyirwani., Winanda, H., Sianturi, D.D., Lestari, A. (2021). Analysis of organic matter content in water and sediment in the coastal waters of Bengkalis Island, Riau Province. IOP *Conf. Series: Earth and Environmental Science.* 934: 1-10.